solution and extracted with $CHCl_3$. The extract was washed with H_2O, dried on K_2CO_3 and distilled *in vacuo* to give the crude compound (XI), whose recrystallization from EtOH gave 100 mg. of XI as colorless plates, m.p. 122~124°. Beilstein test of this compound was negative. IR cm^{-1} (KBr): ν_{NH} 3440, 3340; $\nu_{C=N}$ 1622; δ_{NH} 1642. NMR (τ) (in $CDCl_3$): 7.20 (3H, singlet, C_1-Me); 5.50~6.00 (2H, broad, NH_2); 3.47 (1H, singlet, C_4-H). *Anal.* Calcd. for $C_{10}H_{10}N_2$: C, 75.92; H, 6.38; N, 17.71. Found: C, 75.72; H, 6.42; N, 17.96. Recrystallization of the picrate from EtOH gave yellow needles, m.p. 232~233°(decomp.). *Anal.* Calcd. for $C_{10}H_{10}N_2 \cdot C_6H_3O_7N_3$ (XI): C, 49.62; H, 3.38; N, 18.08. Found: C, 49.75; H, 3.64; N, 17.65.

b) Ammonolysis of 1.0 g. of Xb under the same conditions as the method a) gave 120 mg. of XI. Furthermore, ammonolysis of VI under the same conditions as the method a) also afforded 0.4 g. (25%) of XI. Both specimens were identical with the sample obtained by the procedure a) by mixed melting point test and infrared spectrum.

N-[3-(1-methylisoquinolyl)]acetamide (XIa)——A mixture of 100 mg. of XI with an excess of Ac_2O was heated on a water-bath for 1 hr. After the excess of Ac_2O was removed by distillation *in vacuo*, the residue was basified with 10% aq. NaOH solution and extracted with $CHCl_3$. The extract was washed with H_2O, dried on Na_2SO_4 and distilled. Recrystallization of the resultant residue from *iso*-PrOH gave 70 mg. of XIa as colorless plates, m.p. 202~203°. *Anal.* Calcd. for $C_{12}H_{12}ON_2$ (XIa): C, 71.84; H, 6.04; N, 13.99. Found: C, 72.02; H, 6.24; N, 13.35. IR cm^{-1} (KBr): ν_{NH} 3220; $\nu_{C=O}$ 1658; $\nu_{C=N}$ 1625 (shoulder).

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Kazumi Ogata and Satoru Ishii: Syntheses of Ophthalmic Acid and its Analogues.*1

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In 1956, Waley[1~3] isolated an acidic tripeptide, γ-L-glutamyl-L-α-amino-*n*-butyrylglycine, named ophthalmic acid, and an analogous tripeptide, γ-L-glutamyl-L-alanylglycine, named norophthalmic acid from calf lens. Afterward, he synthesized[4] ophthalmic acid by the reaction of N-benzyloxycarbonyl-L-γ-glutamyl azide with L-α-amino-*n*-butyrylglycine. Enzymatic synthesis of ophthalmic acid was reported by the same authors.[5]

At present, several syntheses of ophthalmic, norophthalmic acids and their analogue are reported. Kermack, *et al.*[6] synthesized DL-norophthalmic acid (γ-DL-glutamyl-DL-alanylglycine) from phthalylglutamic anhydride and alanylglycine. He obtained the optically active acid by the treatment of glutathione with Raney Nickel in poor yield. By using α-*tert* butyl N-benzyloxycarbonylglutamate and (α-amino-*n*-butyryl)glycine *tert* butyl ester, Taschner, *et al.*[7] synthesized ophthalmic acid. Shchukina, *et al.*[8]

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2) *Idem*: *Ibid.*, **67**, 172 (1957).
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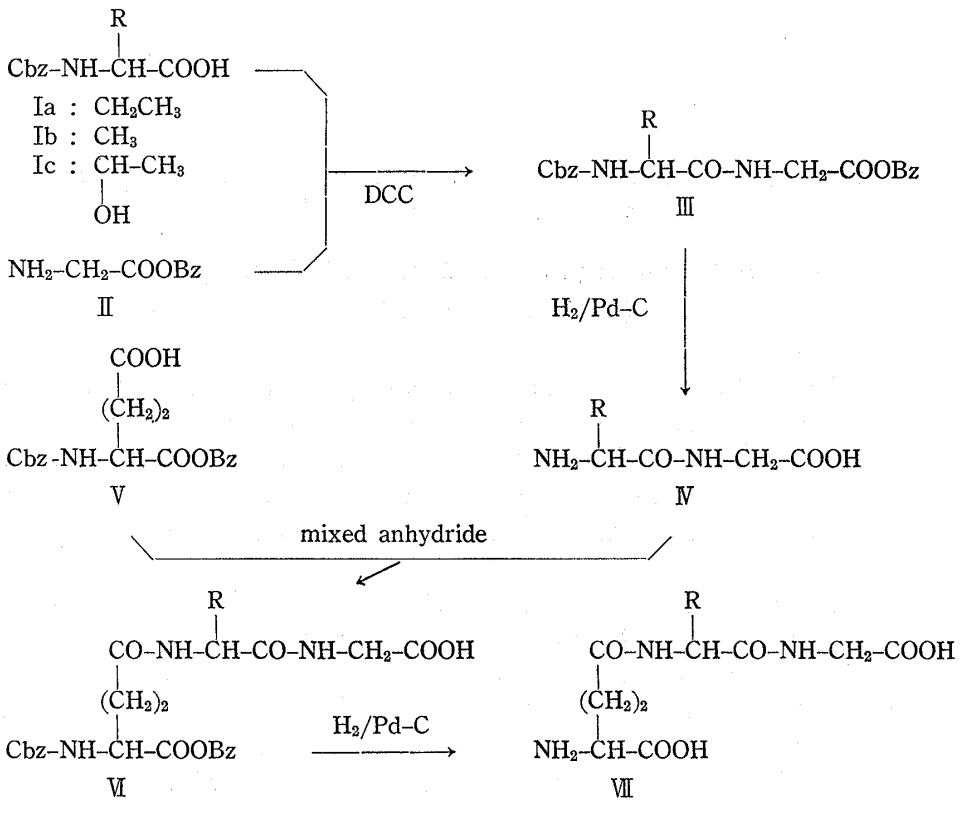
8) L.A. Shchukina, A.L. Jouse: "Peptides," Ed. by G.T. Young, p. 189 (1963), Pergamon Press, London.

prepared the same acid with *p*-nitrobenzyloxycarbonylglutamate and α-amino-*n*-butyrylglycine *p*-nitrobenzyl ester. Losse, *et al.*[9] prepared γ-L-glutamyl-DL-α-amino-*n*-butyrylglycine and γ-L-glutamyl-L-alanylglycine in an active ester method.

The concentration of ophthalmic acid was reported as approximately 0.2 mg. per gram of the lens. This is about one-tenth that of glutathione content in the lens. It seems interest that the two tripeptides, ophthalmic and norophthalmic acid, structurally related to glutathione were found, by the present time, only a few animal tissues, though glutathione distributes in almost tissues of animals and plants. Their physiological functions are still unknown.

In order to explore their biological activities, we are in need of these compounds. Here we wish to record an alternate syntheses of ophthalmic acid and norophthalmic acid. In addition, the threonine analogue of ophthalmic acid was also prepared. Synthetic schema of γ-L-glutamyl-L-α-amino-*n*-butyrylglycine, γ-L-glutamyl-L-alanylglycine and γ-L-glutamyl-L-threonylglycine are illustrated in Chart 1. The synthetic procedures employed are : (1) coupling of α-benzyl N-benzyloxycarbonyl-L-glutamate[10] with dipeptides in a mixed anhydride method, (2) followed by catalytic hydrogenation over a Pd-C catalyst to obtain the γ-L-glutamyl-L-peptides.



Cbz=Benzyloxycarbonyl, Bz=Benzyl, DCC=Dicyclohexylcarbodiimide.

Chart 1.

N-Benzyloxycarbonyl-L-α-amino-*n*-butyric acid (Ia)[4] and N-benzyloxycarbonyl-L-alanine (Ib)[11,12] were reacted with glycine benzyl ester (II) by dicyclohexylcarbodiimide

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to give N-benzyloxycarbonyl-L-α-amino-n-butyrylglycine benzyl ester (IIIa) and N-benzyloxycarbonyl-L-alanylglycine benzyl ester (IIIb)[14] respectively. These compounds were hydrogenated catalytically over a Pd-C catalyst, to give the corresponding dipeptides (IVa)[4] and (IVb)[14~20] respectively. Then, these dipeptides were reacted with α-benzyl N-benzyloxycarbonyl-L-glutamate (V)[10] by the mixed anhydride method to give α-benzyloxycarbonyl-L-glutamyl-L-peptide (VIa) and (VIb) respectively. Finally, catalytic hydrogenation of (VIa) and (VIb) gave γ-L-glutamyl-L-α-amino-n-butyrylglycine (ophthalmic acid) (VIIa) and γ-L-glutamyl-L-alanylglycine(norophthalmic acid) (VIIb) in nearly quantitative yield. These synthetic compounds, (VIIa) and (VIIb), exhibited a single spot on paper chromatography. The acid hydrolysates gave respectively the constituent amino acids in the ratios predicted by theory. All physical constants were quite identical with those reported in the literatures.[4,6,9] We have also prepared the threonine analogue of ophthalmic acid in essentially the same method stated above starting with N-benzyloxycarbonyl-L-threonine (Ic).[13] Examination of the product by paper chromatography and the result of amino acid analysis seems to verify the conclusion that the synthetic compound is homogeneous. Biological examination of those compounds is under progress, these results will be reported in future.

Experimental*3

N-Benzyloxycarbonyl-L-α-amino-n-butyrylglycine Benzyl Ester (IIIa)——N-Benzyloxycarbonyl-L-α-amino-n-butyric acid (8.8 g.), glycine benzyl ester-toluene-p-sulfonate (10.9 g.) and dry triethylamine (3.3 g.) were dissolved in anhydrous tetrahydrofuran (70 ml.). To this ice-cooled solution, dicyclohexylcarbodiimide (6.7 g.) was added. The mixture was stirred for 1 hr. and left overnight. Dicyclohexylurea formed during the reaction was filtered off and the filtrate was evaporated *in vacuo*. The resulting oil was dissolved in AcOEt, which was washed successively with 2% HCl, 4% $NaHCO_3$ and H_2O, and dried over anhydrous Na_2SO_4. The solvent was removed by evaporation. The resulting solid was washed with light petroleum, collected by filtration and recrystallized from a mixture of MeOH and H_2O; yield 9.6 g. (77%). m.p. 115~116°, $[\alpha]_D^{26}$ −26.5°(c=4.0, MeOH). *Anal.* Calcd. for $C_{21}H_{24}O_5N_2$: C, 65.6; H, 6.3; N, 7.3. Found: C, 65.6; H, 6.4; N, 7.2.

N-Benzyloxycarbonyl-L-alanylglycine Benzyl Ester (IIIb)——This compound was prepared from N-benzyloxycarbonyl-L-alanine (11.2 g.), glycine benzyl ester-toluene-p-solfonate (16.9 g.), triethylamine (5.1 g.) and dicyclohexylcarbodiimide (10.3 g.) in essentially the same manner as described above in the preparation of IIIa; yield 15.0 g. (81%), m.p. 111~112°, [lit.[9] m.p. 111°], $[\alpha]_D^{29}$ −23.4°(c=4.0, MeOH). *Anal.* Calcd. for $C_{20}H_{22}O_5N_2$: C, 64.9; H, 6.0; N, 7.6. Found: C, 65.0; H, 6.2; N, 7.5.

N-Benzyloxycarbonyl-L-threonylglycine Benzyl Ester (IIIc)——This compound was prepared from N-benzyloxycarbonyl-L-threonine (8.0 g.), glycine benzyl ester-toluene-p-sulfonate (10.7 g.), triethylamine (3.2 g.) and dicyclohexylcarbodiimide (6.5 g.) in essentially the same manner as described above in the preparation of IIIa; yield 10.0 g. (79%), m.p. 102~104°, $[\alpha]_D^{27}$ −13.1°(c=4.9, MeOH). *Anal.* Calcd. for $C_{21}H_{24}O_6N_2$: C, 63.0; H, 6.0; N, 7.0. Found: C, 62.7; H, 6.2; N, 7.0.

L-α-Amino-n-butyrylgylcine (IVa)——N-Benzyloxycarbonyl-L-α-amino-n-butyrylglycine benzyl ester (6.7 g.) was hydrogenated over a Pd-C catalyst in a mixture of 10% *tert*-BuOH (150 ml.) and AcOH (2 ml.) until evolution of CO_2 ceased. The catalyst was removed by filtration and the filtrate evaporated *in vacuo*. The residual oil was crystallized by addition of MeOH. The product was collected and recrystallized from aqueous MeOH; yield 2.3 g. (83%), m.p. 225~227°(decomp.), $[\alpha]_D^{26}$ +82.5°(c=5.0, H_2O), [lit.[4] m.p. 225°(decomp.), $[\alpha]_D^{16}$ +72], Rf 0.78 (phenol-0.2% NH_4OH). *Anal.* Calcd. for $C_6H_{12}O_3N_2$: C, 45.0; H, 7.6; N, 17.5. Found: C, 45.3; H, 7.7; N, 17.3.

*3 All melting points are uncorrected.

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14) B. F. Erlanger, E. Brand: J. Am. Chem. Soc., **73**, 3508 (1951).
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20) E. Brand, B. F. Erlanger: J. Am. Chem. Soc., **72**, 3314 (1950).

L-Alanylglycine (IVb)——This compound was prepared from N-benzyloxycarbonyl-L-alanylglycine benzyl ester (13.4 g.) by hydrogenation as described above in the preparation of IVa; yield 4.9 g. (93%), m.p. 232～234°(decomp.), $[\alpha]_D^{26}$ +49.5°(c=2.0, H_2O), [lit.[14] $[\alpha]_D^{25}$ +50.6°, lit.[18] $[\alpha]_D^{23}$ +51.3°, lit.[16] $[\alpha]_D^{22}$ +49.1°, lit.[15] $[\alpha]_D^{20}$ +50.2°, lit.[19] $[\alpha]_D^{17}$ +51.44°], Rf 0.70 (phenol-0.2% NH_4OH). *Anal.* Calcd. for $C_5H_{10}O_3N_2$: C, 41.1; H, 6.9; N, 19.2. Found: C, 40.9; H, 7.1; N, 19.2.

L-Threonylglycine (IVc)——This compound was prepared from N-benzyloxycarbonyl-L-threonylglycine benzyl ester (7.0 g.) by hydrogenation as described above in the preparation of IVa; yield 2.5 g. (81%), m.p. 244～246°(decomp.), $[\alpha]_D^{28}$ +53.7°(c=3.7, H_2O), Rf 0.63 (phenol-0.2% NH_4OH). *Anal.* Calcd. for $C_6H_{12}O_4N_2$: C, 40.9; H, 6.9; N, 15.9. Found: C, 41.0; H, 7.2; N, 15.8.

α-Benzyl N-Benzyloxycarbonyl-L-glutamate (V)——This compound was prepared according to the method of Klieger, *et al.*[10]; m.p. 98～99°, $[\alpha]_D^{26}$ −23.8°(c=2.0, MeOH), [lit.[10] m.p. 97～98°, $[\alpha]_D^{25}$ −23.8°]. *Anal.* Calcd. for $C_{20}H_{21}O_6N$: C, 64.7; H, 5.7; N, 3.8. Found: C, 64.8; H, 5.9; N, 4.0.

α-Benzyl N-Benzyloxycarbonyl-L-glutamyl-L-α-amino-*n*-butyrylglycine (VIa)——A mixed anhydride was prepared in an ice-bath from α-benzyl N-benzyloxycarbonyl-L-glutamate (4.6 g.) in anhydrous tetrahydrofuran (30 ml.) with triethylamine (1.3 g.) and ethyl chloroformate (1.4 g.). This solution was added slowly with stirring to a chilled solution of L-α-amino-*n*-butyrylglycine (2.0 g.) and triethylamine (1.3 g.) in H_2O (10 ml.). The mixture was stirred in an ice-bath for 1 hr. and at room temperature for 2 hr. The solvent was evaporated *in vacuo*. The residue was acidified with 1*N* HCl and the resulting precipitate was collected by filtration, washed successively with H_2O and ether, and recrystallized from MeOH; yield 4.1 g. (64%), m.p. 178～179°, $[\alpha]_D^{28}$ −25.0°(c=3.3, AcOH). *Anal.* Calcd. for $C_{26}H_{31}O_8N_3$: C, 60.8; H, 6.1; N, 8.2. Found: C, 61.0; H, 6.4; N, 8.2.

α-Benzyl N-Benzyloxycarbonyl-L-glutamyl-L-alanylglycine (VIb)——This compound was prepared from α-benzyl N-benzyloxycarbonyl-L-glutamate (3.7 g.) and L-alanylglycine (1.5 g.) by the mixed anhydride method as described above in the preparation of VIa; yield 3.2 g. (64%), m.p. 191～192°, $[\alpha]_D^{29}$ −26.9°(c=2.0, AcOH). *Anal.* Calcd. for $C_{25}H_{29}O_8N_3$: C, 60.1; H, 5.9; N, 8.4. Found: C, 60.3; H, 6.0; N, 8.4.

α-Benzyl N-Benzyloxycarbonyl-L-glutamyl-L-threonylglycine (VIc)——This compound was prepared from α-benzyl N-benzyloxycarbonyl-L-glutamate (3.7 g.) and L-threonylglycine (1.8 g.) by the mixed anhydride method as described above in the preparation of VIa. It was recrystallized from EtOH; yield 3.3 g. (62%), m.p. 148～149°, $[\alpha]_D^{28}$ −16.0°(c=2.0, AcOH). *Anal.* Calcd. for $C_{26}H_{31}O_9N_3$: C, 59.0; H, 5.9; N, 7.9. Found: C, 58.8; H, 6.0; N, 8.0.

γ-L-Glutamyl-L-α-amino-*n*-butyrylglycine(ophthalmic acid) (VIIa)——α-Benzyl N-benzyloxycarbonyl-L-glutamyl-L-α-amino-*n*-butyrylglycine (3.0 g.) was hydrogenated over a Pd-C catalyst in a mixture of 10% (v/v) *tert*-BuOH (100 ml.) and AcOH (2 ml.) until the evolution of CO_2 ceased. The catalyst was removed by filtration and the filtrate was evaporated *in vacuo*. The residual oil precipitated by addition of Me_2CO was collected and recrystallized from H_2O by addition of Me_2CO; yield 1.2 g. (68%), m.p. 177～179° (decomp.), $[\alpha]_D^{26}$ −28.4°(c=2.1, H_2O), [lit.[4] $[\alpha]_D^{20}$ −29], Rf 0.59 (phenol-0.2% NH_4OH), single ninhydrin positive spot. Amino acid ratios in an acid hydrolysate $Glu_{1.00}$α-amino-*n*-$but_{1.01}Gly_{0.98}$ (average recovery 92%). *Anal.* Calcd. for $C_{11}H_{19}O_6N_3 \cdot H_2O$: C, 43.0; H, 6.9; N, 13.7. Found: C, 43.2; H, 7.3; N, 13.8.

γ-L-Glutamyl-L-alanylglycine (norophthalmic acid) (VIIb)——This compound was prepared from α-benzyl N-benzyloxycarbonyl-L-glutamyl-L-alanylglycine (5.0 g.) by hydrogenation as described above in the preparation of VIIa; yield 2.0 g. (71%), m.p. 197～199°(decomp.), $[\alpha]_D^{26}$ −29.8°(c=2.4, H_2O), Rf 0.51 (phenol-0.2% NH_4OH), single ninhydrin positive spot. Amino acid ratios in an acid hydrolysate $Glu_{1.00}Ala_{1.08}Gly_{0.93}$ (average recovery 93%). *Anal.* Calcd. for $C_{10}H_{17}O_6N_3 \cdot \frac{1}{2}H_2O$: C, 42.2; H, 6.4; N, 14.8. Found: C, 41.8; H, 6.6; N, 14.8.

γ-L-Glutamyl-L-threonylglycine (VIIc)——This compound was prepared from α-benzyl N-benzyloxycarbonyl-L-glutamyl-L-threonylglycine (2.5 g.) by hydrogenation as described above in the preparation of VIIa; yield 1.0 g. (68%), $[\alpha]_D^{30}$ −16.5°(c=2.9, H_2O), Rf 0.38 (phenol-0.2% NH_4OH), single ninhydrin positive spot. Amino acid ratios in an acid hydrolysate $Glu_{1.02}Thr_{1.00}Gly_{0.98}$ (average recovery 80%). *Anal.* Calcd. for $C_{11}H_{19}O_7N_3 \cdot \frac{1}{2}H_2O$: C, 42.0; H, 6.4; N, 13.4. Found: C, 42.2; H, 6.3; N, 13.3.

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